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Prediction and Diagnosis of Early *Pseudomonas aeruginosa* Infection in Cystic Fibrosis: a Follow-Up Study

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Immunoglobulin G (IgG) antibodies to *Pseudomonas aeruginosa* surface antigens in serum were estimated by enzyme-linked immunosorbent assay for all patients from whom *P. aeruginosa* was isolated for the first time during a study period of 3 years (33 patients). The titer of IgG antibodies was greater than control values at or up to 24 months before the first isolation of *P. aeruginosa* in 24 patients. Another five patients had titers that were within the control range before isolation of *P. aeruginosa* but increased to above the control range within the following 2 months. In these 29 patients, continuing intermittent isolations of *P. aeruginosa* were accompanied by further increases in titer. The presence of a systemic immune response above the control range indicates tissue invasion and hence infection. Four patients were deemed to have no infection: one or two isolations of *P. aeruginosa* were accompanied by no increase in specific antibodies to above the control range throughout the entire study period. Fifteen patients received intravenous antipseudomonal chemotherapy. Eradication of the organism and a return of titer to control values, suggesting complete removal of the organisms, occurred in 5 patients, while continued isolations and only a partial decrease in titer occurred in 10 patients. The 15 patients who received treatment improved clinically, in contrast to untreated patients, whose clinical state worsened during the study period. Continuous steroid treatment, given to two patients, was accompanied by a dramatic decrease in both serum IgG concentration and titer, despite continuing intermittent isolations of *P. aeruginosa*. These results confirm and extend our earlier finding that this assay appears to detect *P. aeruginosa* infection at a very early stage and helps in differentiating between early infection and harmless colonization. It also appears to be a useful monitor of the progress of infection and the response to intravenous antibiotic treatment in these early stages of infection, before any clinical changes are sufficiently large to be detected, in patients who were not on continuous steroid therapy. The effect of steroid treatment on the immunological response and clinical outcome of patients with early *P. aeruginosa* infection requires further investigation.

Lung infection by *Pseudomonas aeruginosa* remains a major cause of morbidity and subsequent mortality in cystic fibrosis (CF) patients (12, 16). Despite the advent of effective anti-pseudomonal antibiotics, once the organism is established in the lungs it is extremely difficult to eradicate. Interpretation of the bacteriological results of sputum cultures can be difficult, particularly when several organisms are isolated. When present in sputum cultures, organisms may be considered indicative of harmless, nonpathogenic colonization or of infection. In addition, two other problems may be encountered: organisms present in low numbers may be overlooked in mixed cultures, and in the absence of chronic pulmonary infection, many CF patients produce little or no sputum, so that respiratory cultures are based on throat or cough swabs. The results of throat and cough swab cultures may give an inaccurate picture of the bacteriology in the lower respiratory tract. We have previously described an enzyme-linked immunosorbent assay (ELISA) that detected free immunoglobulin G (IgG) antibodies to cell surface antigens of *P. aeruginosa* in serum. The contribution of cross-reacting antibodies (directed primarily against other gram-negative organisms) to the assay was negligible (5). Titers in CF and non-CF patients with no known *P. aeruginosa* infection were low, while high titers were associated with chronic *P. aeruginosa* infection and correlated with poor clinical state (3).

In this paper we present our findings on the use of this assay in a prospective study on all CF patients from whom *P. aeruginosa* was isolated for the first time during a study period of 3 years.

MATERIALS AND METHODS

Patients. The patients included in this study were all CF patients from whom *P. aeruginosa* was isolated from respiratory cultures for the first time during a study period of 3 years. The patients attended the pediatric and adult CF clinics in our hospital. All had characteristic features of the disease and had had at least one positive sweat test (7). Their age range was 1 month to 27 years, and there were 20 females and 14 males. Serum samples were taken surplus to requirements for other tests.

Overall clinical status was measured by the Shwachman score (17). A maximum of 25 points were awarded for each of the following: nutrition, general activity, physical examination, and chest X-ray film. A worsening condition is indicated by a lower score. Chest X-ray films were assessed by the Chrispin-Norman score (6). The possible scores were 0 to 38, with an increasing score indicating increasing abnormality.

Microbiology. Sputum or cough swab samples were collected from all patients. The number of specimens per patient varied from 5 to 30 annually during the study period. The proportion of specimens from each patient that were sputum samples was 66 to 100% (mean, 74 ± 10%). Patients with an unproductive cough were asked to cough, and the back of the oropharynx was touched with an alginate swab

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and used as a cough swab. Sputum was collected from patients with a productive cough after physiotherapy and processed as follows.

Sputum samples. Sputum was deemed suitable for culture if it was purulent or mucopurulent. The whole sample was digested at 37°C for 30 min with an equal quantity of dithiothreitol (Sputasol; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) to give a 1:2 dilution. The following plates were inoculated with a 10-μl sample of digested sputum. (i) Sabouraud (Oxoid Ltd.) agar containing gentamicin (60 μg/ml; Nicholas Laboratories Ltd., Slough, Berkshire, United Kingdom) and colistin (13 μg/ml; Pharmax, Bexley, Kent, United Kingdom) was used for selective isolation of yeasts and fungi. (ii) Mannitol salt agar (Oxoid Ltd.) containing 7.5% sodium chloride was used for selective isolation of *Staphylococcus aureus*. (iii) Heated blood agar (Oxoid Ltd.) containing bacitracin (10 μg/ml; Calbiochem, c/o Cambridge Bioscience, Cambridge, United Kingdom) was incubated anaerobically to isolate *Haemophilus* spp. (iv) Columbia blood agar (Oxoid Ltd.) was incubated in 5% CO_2 as a general culture medium. (v) For the isolation of pseudomonads and differentiation of colonial variants, the digested sputum was diluted 1:50 in peptone water (Oxoid Ltd.) to give a final dilution of 1:100. Ten microliters of this dilution was inoculated onto a 12-cm^2 plate (RossLab, Macclesfield, Cheshire, United Kingdom) filled with heated blood agar containing bacitracin (10 μg/ml). The inoculum was spread over one-third of the surface of the plate with a sterile swab. Two stroke lines were made to the center third of the plate and spread with another swab. This was repeated for the lower third as before. The use of the larger plate and this spreading technique improve the separation of colonies for further serological typing, antibiotic susceptibility testing, and identification. All plates were examined after 24 and 48 h of incubation. Isolates showing different colonial morphology after 48 h of incubation were selected and tested for susceptibility to antibiotics. The strains of *Pseudomonas* isolated were identified by means of the API 20NE system of biochemical reactions (API Products Ltd., Basingstoke, Hampshire, United Kingdom) and serotyped when necessary with the international serotyping antiserum (Difco Laboratories, East Molesley, Surrey, United Kingdom). Antibody susceptibility testing of pseudomonads was performed by using the breakpoint technique of Mast Laboratories, Bootle, Merseyside, United Kingdom. Adatabs of individual antibiotics were mixed with DST agar (Oxoid Ltd.) to give the following concentrations: gentamicin, tobramycin, and ciprofloxacin, 2 and 8 μg/ml; ceftazidime, 4 and 16 μg/ml; amikacin, 8 and 32 μg/ml; azlocillin and piperacillin, 16 and 64 μg/ml; and colistin, 4 μg/ml. After incubation for 24 h at 37°C, isolates were deemed resistant if they grew in neither concentration and partially resistant if they grew only at the lower concentration of antibiotic, in accordance with the Mast Laboratories handbook. The antibiotic susceptibility of non-*Pseudomonas* isolates was tested by using a conventional disk method: disks of the above eight antibiotics (Oxoid Ltd.) were placed on a lawn of a single organism. Growth after 24 h of incubation at 37°C was compared with that of the standard strain (NCTC 10662). (vi) *Pseudomonas cepacia* selective medium (Mast Laboratories) was inoculated from the digested sputum (1:100 dilution). The plate was examined daily for 3 days. Suspect colonies were identified by the API 20NE system of biochemical reactions (API Products Ltd.). *P. cepacia* was never isolated from this group of patients.

Cough swabs. Cough swabs were inoculated directly on Columbia blood agar, incubated at 5% CO_2, and onto two heated blood agar plates containing bacitracin (10 μg/ml) for aerobic and anaerobic cultures, respectively.

Serum. All samples were stored at −20°C until immediately before use.

ELISA. IgG antibodies to *P. aeruginosa* cell surface antigens were measured by ELISA as described previously (3, 5). Seven strains of *P. aeruginosa* with serotypes 1, 3, 6, 9, 10, and 11 and a strain that could not be serotyped were used (11). These strains constituted the majority (86%) of isolates from CF patients in our unit (5).

Briefly, each serotype was grown overnight on blood agar plates at 37°C, removed in 10 ml of phosphate-buffered saline (PBS), and then washed three times in PBS. Polystyrene microtiter plates (Immulon grade A; Dynatech) were coated overnight at 4°C with a suspension of cells (10^{10} CFU/ml) in PBS containing 0.3% (vol/vol) methylglyoxal (Sigma). Bound cells were fixed with glutaraldehyde (0.5%, vol/vol) for 30 min at room temperature, and unbound sites were blocked by 1% bovine serum albumin (Sigma Chemical Co. Ltd., Poole, Dorset, United Kingdom) in PBS. A single serotype was used on each plate.

Test serum was diluted 1:1,000 or 1:10,000 in PBS–1% bovine serum albumin and incubated in triplicate for 75 min at room temperature. After washing, goat anti-human IgG (γ-chain specific) conjugated to horseradish peroxidase (Zymed Laboratories Inc., South San Francisco, Calif.), diluted 1:2,000, was incubated for 2 h at room temperature. The enzyme substrate solution contained orthophenyldiamine (0.5 mg/ml) (Sigma Chemical Co.) in citrate phosphate buffer (2.43 ml of citric acid [0.1 M], 2.57 ml sodium phosphate [0.2 M], and 5.0 ml of distilled water). The reaction was stopped after 2 min by adding H_2SO_4 (0.1 M) (B.D.H. Ltd., Poole, Dorset, United Kingdom) and the A_{492} was read in a Dynatech plate reader (Dynatech Laboratories Ltd.). Wells containing no adsorbed antigen or no test serum were included in each plate and gave an absorbance of <0.10. A standard curve on each plate was used to calculate the titer. This covered the full range of absorbance between 0.1 and approximately 1.8, equivalent to a serum titer of 140 and above (3, 4).

The coefficient of variation for intra-assay measurements (10) of a single serotype was 1 to 5%. The coefficient of variation of interassay measurements (10) of the sum titer was 4 to 10%, which is in agreement with the variation of the sum titer obtained by multiplying the maximum intra-assay variation of 5% for a single serotype by $\sqrt{7}$.

Statistics. Student's *t* test (10) and Kendall's rank correlation test (18) were used for analysis. The level of significance was 0.05 for two-tailed analysis.

RESULTS

Diagnosis of the beginning of *P. aeruginosa* infection. *P. aeruginosa* was isolated for the first time from the respiratory tracts of 33 CF patients during a 3-year study period.

Twenty-four of these patients had an antipseudomonal IgG antibody titer between 304 and 990 either at the first isolation of *P. aeruginosa* or up to 24.5 months before this event (Fig. 1). Previous work in our laboratory has shown that in patients without CF who have had no known infection by *P. aeruginosa*, the upper limit of titers with statistical confidence limits of 99.9% (mean plus 3.291 times the standard deviation [SD]) was 270 (3, 5). Hence, these 24 patients had titers greater than the control range up to 2 years before isolation of *P. aeruginosa* from the respiratory

TABLE 1. Titer of IgG to *P. aeruginosa* in patients with CF before and after the beginning of intermittent isolation from the respiratory tract[a]

Patient no.	Age (yr)	Sex	Before *P. aeruginosa* isolation		After *P. aeruginosa* isolation	
			Time before first isolation (mo)	IgG titer	Time after first isolation (mo)	IgG titer
1	10	Female	15	<140	1	410
			7	<140		
			0.5	<140		
2	9	Female	11	<160	8	300
					17	720
3	2	Male	20	250	1.25	265
					1.5	1,000
4	1	Male	12	<240	0.75	330
					2	520
5	9	Male	20	220	0.25	760

[a] IgG titer refers to the sum titer in serum samples to seven strains with serotypes 1, 3, 6, 9, 10, and 11 and a strain that could not be serotyped.

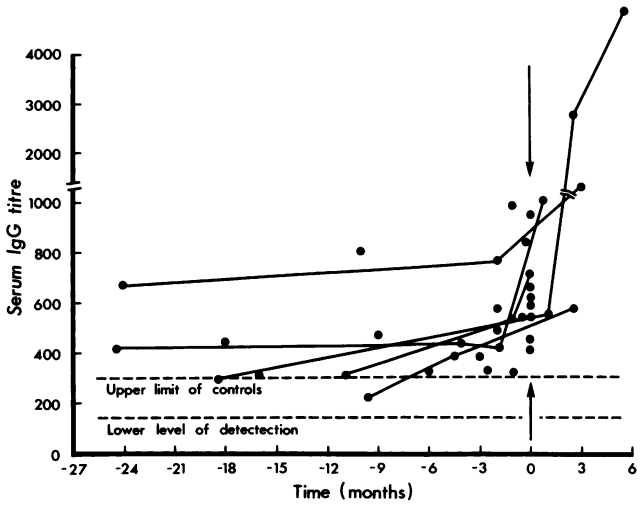

FIG. 1. Titer of IgG antibody to *P. aeruginosa* in serum of CF patients before and after the first isolation from the respiratory tract. Single serum samples were available for 19 patients and serial samples were available for 5 patients. The titer is the sum of individual titers of antibody to strains with serotypes 1, 3, 6, 9, 10, and 11 and an untypeable strain. The arrows indicate the time of first isolation of *P. aeruginosa*. The upper limit of controls (300) is the statistical confidence limit of 99.9% (mean + 3.291 times SD) plus 10% interassay error.

tract. There was no correlation between antipseudomonal IgG titer and length of time before isolation of *P. aeruginosa* for the entire group of patients. When serial samples were available from individual patients before isolation of *P. aeruginosa*, they showed an increase with time, but the rate of change varied in different patients (Fig. 1).

Another five patients had titers within the control range at times during the 20 months before the first isolation of *P. aeruginosa*, but during the 2 months following the first isolation, the titer had increased to greater than control values (300 to 1,000, Table 1).

The presence of a specific systemic immune response, above the normal range, was considered to imply tissue invasion and to indicate that the patients were either suffering or about to develop early infection.

Finally, *P. aeruginosa* was isolated between one and five times from fortnightly or monthly respiratory cultures from four CF patients, during study periods of 10 to 36 months. IgG titers in serum before (0 to 14 months) and after (2 to 7 months) *P. aeruginosa* isolation were within the control range in these four patients (Table 2). The IgG concentration in serum of these patients was also within the normal range throughout the study period. The absence of a systemic immune response was therefore considered to imply that these four patients were not infected but rather transiently colonized.

Effect of i.v. antipseudomonal therapy. Fourteen of the 29 patients with putative *P. aeruginosa* infection did not receive treatment. They were clinically well and developed no new signs or symptoms of infection. *P. aeruginosa* continued to be isolated intermittently (38 to 71% positive specimens). Serial serum samples were available from seven patients. The titer increased from an initial value of 360 to 720 to 2,200 to 9,400 approximately 1 year later (Fig. 2A), and their clinical state deteriorated slightly during this period (Table 3).

Fifteen of the 29 infected patients were given intravenous (i.v.) antipseudomonal treatment once or twice within 1 to 4 months of the beginning of intermittent isolations of the organism. Treatment was prompted by the development of new minor signs and symptoms of infection, such as a new cough or a slight increase in the severity of cough and feeling slightly unwell. Clinical parameters indicated a slightly worse initial clinical state in patients who were given such treatment than in those who were not, but no difference was statistically significant (Table 3).

There were two types of response to treatment. *P. aeruginosa* continued to be isolated intermittently (26 to 76%

TABLE 2. Titer of IgG antibody to *P. aeruginosa* in patients with CF before and after transient isolation from the respiratory tract

Patient no.	Age (yr)	Sex	No. of cultures positive/no. tested	Sampling duration (mo)	Before *P. aeruginosa* isolation		After *P. aeruginosa* isolation			IgG concn in serum (g/liter)	
					IgG titer	mo	IgG titer	mo	mo without positive sample	Actual	Normal range
1	1	Male	1/17	10	<140	1	<140	0	14	3.32	3–13
							<140	6		3.32	3–13
2	8	Male	3/25	27	<240	10.5	<160	16	22	9.63	5–16
3	6	Male	5/61	36	NS[a]	NS	<170	11	17	8.6	5–16
4	4	Female	1/13	11	<160	16.5	<190	7	14	5.08	5–16
					<170	3				8.55	5–16

[a] NS, No sample available.

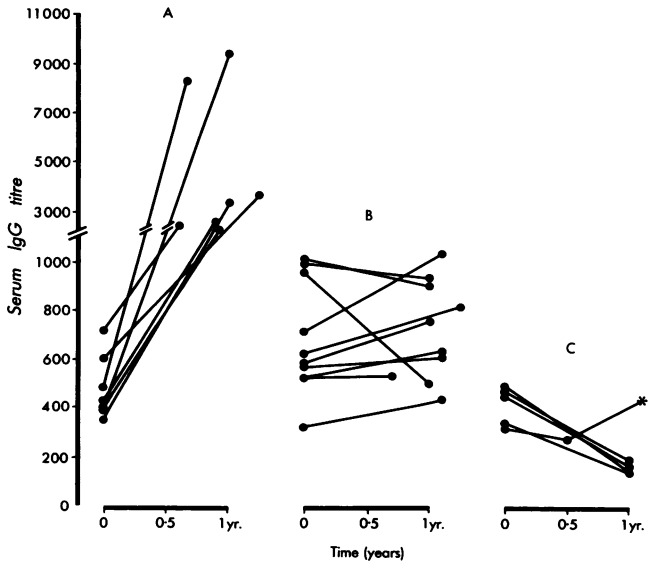

FIG. 2. Longitudinal studies on CF patients with early *P. aeruginosa* infection. *P. aeruginosa* was isolated intermittently for 1 to 10 weeks before time zero. i.v. treatment, if applicable, began at time zero. Intermediate values have been omitted for the sake of clarity. (A) No i.v. treatment given, intermittent *P. aeruginosa* isolation continued; (B) 2 weeks of i.v. treatment given, *P. aeruginosa* continued to be isolated intermittently; five patients received tobramycin and piperacillin, three received tobramycin and azlocillin, and two received ceftazidime; (C) 2 weeks of i.v. treatment given, *P. aeruginosa* not isolated except when marked (*). Four patients received tobramycin and piperacillin, and one received ceftazidime. The lower level of detection was 140, and the upper limit for controls (99.9% confidence limits) was 270 (mean + 3.291 times SD).

positive specimens) from 10 of 15 patients. In spite of a transient increase in titer after half the courses of antibiotic treatment, within approximately 1 month the titer decreased, although it remained greater than control levels. Within the next few months after treatment, however, titers began to increase again to reach the starting level within approximately 10 to 13 months (Fig. 2B). All patients received 2 weeks of i.v. antibiotics: five received tobramycin and piperacillin, three received tobramycin and azlocillin, and two received ceftazidime.

Treatment was followed by no further isolations of *P. aeruginosa* from the other five patients. The titer decreased to within the control range during the 14 to 32 months following treatment in four of these five patients (Fig. 2C). The clinical state of these four patients improved slightly during the study period (Table 3). One patient remained free of *P. aeruginosa*, with titers within the range of control titers, for 5 months following i.v. therapy, but *P. aeruginosa* was then isolated intermittently throughout the rest of the study period. Serum samples taken 9 and 21 months after i.v. therapy showed that titers had risen to above control values (535 and 590, respectively). i.v. treatment was continued for 2 weeks in all cases: four patients received tobramycin and piperacillin, and one patient received ceftazidime.

There was no difference between patients in whom infection was and was not eradicated in the following characteristics: age, sex, previous organism isolation, antibiotic treatment, and length of time of raised titers or of *P. aeruginosa* isolation before treatment. There was no statistically significant difference between the initial titer, the Shwachman

TABLE 3. Changes in clinical and immunological parameters of CF patients with early *P. aeruginosa* infection and effect of i.v. treatment[a]

Treatment group (no. of patients)	Age (yr)		Schwachman score [range (mean ± SE)]		Crispin-Norman score [range (mean ± SE)]		$FEV_{1.0}$ % predicted [range (mean ± SE)]		No. of cultures tested (% positive)
	Range	Mean ± SE	Time zero	1 yr later	Time zero	1 yr later	Time zero	1 yr later	
No i.v. treatment (7)	7–26	13.8 ± 2.3	80–95 (87.5 ± 2.5)	65–95 (82 ± 5)	0–10 (5.5 ± 1.9)	1–12 (7 ± 2.3)	58–145 (96 ± 13)	58–96 (77 ± 6)	10–30 (38–71)
i.v. treatment									
Total (15)	1–25	11.6 ± 3.0	50–90 (69 ± 5)	70–95 (82 ± 3)	1–23 (12 ± 2)	3–11 (8 ± 1)	43–100 (77 ± 7)	47–128 (87 ± 8)	
P. aeruginosa persisted (10)	1–25	12.4 ± 2.5	50–70 (67 ± 6)	70–95 (83 ± 3)	7–23 (15 ± 4)	8–11 (10 ± 0.6)	43–96 (68 ± 10)	47–100 (75 ± 11)	16–30 (26–76)
P. aeruginosa eradicated (5)	4–22	10.4 ± 3.5	60–80 (73 ± 7)	75–90 (80 ± 5)	1–15 (7 ± 4)	3–9 (5 ± 2)	68–100 (85 ± 8)	70–128 (100 ± 11)	10–22 (0)

[a] *P. aeruginosa* was isolated intermittently for 1 to 10 weeks before time zero. i.v. treatment, if applicable, began at time zero. Five patients received tobramycin and piperacillin, three received tobramycin and azlocillin, and two received ceftazidime in the group in which *P. aeruginosa* persisted. Four patients received tobramycin and piperacillin and one received ceftazidime in the other group. Treatment continued for 2 weeks in all cases.

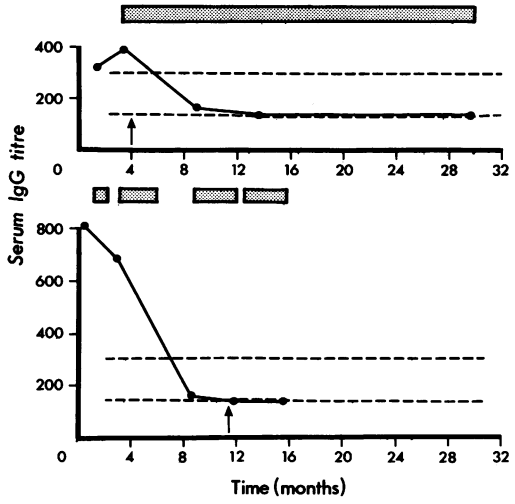

FIG. 3. Effect of steroid treatment on titer of IgG antibody in patients with CF. The IgG titer in serum is the sum of individual titers to strains with serotypes 1, 3, 6, 9, 10, and 11 and a strain that could not be serotyped. The dashed lines define the lower limit of detection (bottom) and the upper limit of controls (top) (99.9% confidence limits). The hatched areas indicate continuous treatment with prednisone. The arrow shows the start of intermittent isolations of *P. aeruginosa* from the respiratory tract.

score (17), the Chrispin-Norman X-ray score, or the $FEV_{1.0}$ % predicted (forced expiratory volume achieved in 1 s expressed as a percentage of that predicted by height and sex) for the two groups of patients. At the end of the study period, clinical parameters of treated patients had improved slightly to become broadly similar to those of untreated patients. Titers, which initially were not significantly different between treated and untreated patients, were finally significantly lower in both treated groups than in untreated patients ($P < 0.05$).

Effect of continuous steroid treatment. Two CF patients had raised titers (385 and 840, respectively) before the first isolation of *P. aeruginosa*. They then began continuous steroid treatment (prednisone), as they had significant bronchospasm which was only partially responsive to bronchodilators, but they were otherwise in excellent general condition. Continuous steroid treatment was accompanied by a rapid decline in titer to the lower level of detection in both patients (titer, 140) (Fig. 3) and by a decrease in IgG concentration in serum from the middle (7.5 and 10.2 g/liter) to the lower end of the normal range (5.1 and 5.0 g/liter). During the following 9 to 22 months of continuous steroid treatment, the titer and the IgG concentration remained at these low levels in both patients, despite intermittent isolations of *P. aeruginosa* throughout the study period.

DISCUSSION

The results presented here are the outcome of a prospective longitudinal study of the measurement of anti-pseudomonal IgG antibodies in all the CF patients in our hospital from whom *P. aeruginosa* was isolated for the first time during a period of 3 years.

An increase in IgG titer in serum above the range of control values was detected up to 24.5 months before *P. aeruginosa* was present in sufficient numbers to be isolated from the respiratory tract of 24 of 33 CF patients. There was no correlation between titer and time before isolation of *P. aeruginosa* over the whole group of 24 patients, but when serial samples were available from individual patients, they revealed a gradual increase in IgG titer with time, suggesting continuous antigen presence. We have previously reported that IgG titers measured by this assay were low in both CF patients and other pediatric patients who had no history of *P. aeruginosa* infection (3, 5). Furthermore, the contribution of cross-reacting antibodies, directed primarily against other bacteria, to this assay was negligible (5). There were, however, a minority (5 of 29) of patients from whom the organism was isolated at a very early stage of infection; antibody titers were normal before the first isolation but increased within the following 6 weeks.

P. aeruginosa was subsequently isolated intermittently (26 to 76% positive specimens) from all 29 of these patients, and isolation was accompanied by further increases in titer that were fast or slow in different patients. This increase in titer presumably reflects the continuous and increasing bacterial load and signifies early *P. aeruginosa* infection in all 29 patients.

In contrast, *P. aeruginosa* was isolated once or twice from four patients but no systemic immune response was detected over 1 to 3 years. This implies either that the isolates were transient or that the local immune response was completely adequate and prevented any significant tissue invasion. The total IgG concentration in serum of these patients was within the normal range throughout the study period, so they did not have a low humoral immune response. Even if the serotype of organisms from these patients was not among those included in the ELISA, antibodies directed against the outer membrane proteins would be detected, and these are highly conserved in *P. aeruginosa* (8, 13).

P. aeruginosa infection was apparently eradicated in some patients after treatment: the organism has not been isolated for 1 to 3 years. The return of titers to the lower end of control values implies removal of the antigenic stimulus, i.e., *P. aeruginosa*, and therefore by inference the removal of the cause of infection. In one of these patients, however, infection has subsequently recurred: the organism was isolated after an interval of 5 months, and titers increased above the control range, confirming the recurrence of infection.

In other patients, however, pseudomonal infection remained despite treatment. There was no difference between these patients and those in whom the infection was apparently eradicated in any of the clinical parameters examined. There was no clear-cut difference in initial titers between the two groups of patients as a whole.

Patients who were treated with i.v. antipseudomonal antibiotics were initially in a slightly worse clinical state than those who were not treated. One year later, after treatment, this situation was reversed. Changes in titer showed the same trend of worsening infection among untreated patients and improvement in treated patients. None of the changes in clinical parameters or their starting values were statistically significant, nor was there a significant correlation between titer and clinical parameters. Pseudomonal infection in these patients was at a very early stage, so it may not have been detected by these relatively crude measures of infection. Furthermore, the clinical parameters will also be affected by the severity of previous nonpseudomonal infection, which differs in different patients. In chronic infection, during which the changes are larger and are due solely to *P. aeruginosa*, we have shown a significant correlation between titer and both Chrispin-Norman X-ray score and Shwachman score (3).

Despite the lack of statistical significance, the improve-

ment in a group of patients who were initially iller is encouraging. The possibility of eradicating the infection would have an even greater effect on the long-term prognosis and survival of patients and is in complete contrast to established clinical experience with chronic infection.

Continuous steroid treatment given to two patients with elevated titers was accompanied by a sharp decrease in titer to the lower level of detection and in total IgG concentration in serum to the bottom of the normal range. Other workers have shown that CF patients given prednisone for 4 years had significantly lower IgG concentrations than untreated patients (1). It therefore seems that there are essentially no IgG antipseudomonal antibodies in the serum of these patients, and hence none are available for transduction through the lungs. This may be beneficial for the patient: the formation of immune complexes, and accompanying tissue damage, is less likely in the absence of high levels of antibodies. Alternatively, if other components of the systemic or local humoral immune response are either initially inadequate or depressed by steroid treatment, then the effects of extracellular pseudomonal virulence factors will not be inhibited by antibody binding. Continuous steroid treatment may also affect pulmonary clearance of organisms (2, 15). Further longitudinal studies with patients receiving continuous steroid treatment are needed to clarify these points.

These results confirm and extend our previous finding that this ELISA gives a very early diagnosis of pulmonary infection by *P. aeruginosa* (3, 5), with the notable exception of patients receiving continuous steroid treatment. It has been suggested that many CF patients initially become colonized with *P. aeruginosa*, which is followed some time later by infection. The host immune response has been used to identify infected patients (9, 14). The counterimmunoelectrophoresis techniques used in the past are now replaced by the more sensitive ELISA (14). The time at which infection is determined to have begun is dependent on the method of measurement used. It is also somewhat arbitrary: the level at which harmless colonization, which may cause a slight increase in systemic antibody levels, changes into early infection may be open to debate. What is perhaps more relevant is the ability to delay or prevent the progression of infection.

The possibility of eradicating *P. aeruginosa* infection by i.v. treatment, which we have now demonstrated, has considerable implications for the prognosis of patients with raised titers from whom the organism is isolated intermittently. Carried to its logical conclusion, i.v. treatment of patients who have raised titers but from whom *P. aeruginosa* has not yet been isolated should delay or prevent the appearance of the organism in samples. We are currently investigating the effect of antipseudomonal treatment, on the basis of raised titer alone, on patients both before and after isolation of *P. aeruginosa*. The effect of such early treatment on the appearance of resistant strains and the rate of relapse (i.e., reappearance of infection) should also be clarified.

ACKNOWLEDGMENTS

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